opic: Biosynthesis of Silver Nanoparticles by *A. moluccana leaf* and *p. pastoris* By Egesa Dan Supervisor: Prof. Li qingbiao Xiamen University

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MASTER DEGREE THESIS

Biosynthesis of Silver Nanoparticles by sundried *Aleurites moluccana* leaf and yeast *pichia pastoris*

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DEPARTMENT OF CHEMICAL AND BIOCHEMICAL ENGINEERING
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CERTIFICATION

I, Professor , hereby certify that I have read this manuscript and recommend for acceptance by the Xiamen University a dissertation entitled "Biosynthesis of silver nanoparticles by sundried *Aleurites moluccana* leaf and yeast *pichia pastoris*" in fulfillment of degree of Master of Engineering at Xiamen University, People's Republic of China.

Signed………………

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Date…………………

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ORIGINAL STATEMENT

The research described in this Master of Engineering thesis was conducted under the supervision of Professor Professor Li Qingbiao_at the Department of Chemical and Biochemical Engineering, Xiamen University. I hereby declare that the work submitted is my own and that appropriate credit has been given where reference has been made to the work of others. I also confirm that it has not been previously or concurrently submitted for any other degree, diploma or any other qualifications at Xiamen University, P.R China or other institutions.

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Date:

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All thanks and praise to God Almighty for the gift of life and grace to do this work without which it wouldn't be possible. This work is dedicated to Him.

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ABSTRACT

Because the properties of silver nanoparticles depend so closely on their size, size distribution and morphology, controlling the size and shape of silver nanoparticles is of great interest to researchers today. In this study, we focus on the control of size of silver nanoparticles biologically synthesized using *aleurites moluccana* leaf broth and *pichia pastoris*, as reducing agents. The effect of reaction temperature, rotate rate, the ratio between biomass concentration to silver ions, and pH on the synthesis process of silver nanoparticles was investigated. The formation of nanoparticles was understood and monitored by UV–Vis spectrometer, transmission electron microscopy (TEM), scanning electron microscopy (SEM), and Fourier transform infrared spectrometer (FTIR).

Using *A.moluccana* leaf broth as reducing agent, when the reaction temperature was fixed at 80 ^{0}C, the mean particle diameter was 31.1 nm, increase in reaction temperature to 100 ^{0}C, led to reduction of the mean particle diameter to 13.2 nm. Increasing the rotate rate led to steady reduction in absorbance and a blue shift in the absorbance peak, there was reduction in particle size from 26.3 nm to 25.5 nm with increase in rotate rate from 10 rpm to 30 rpm respectively. While increasing biomass concentration led to increase in absorbance and a blue shift in the position of the spectrum suggesting reduction in particle size. In addition, pH was found to have a great impact on the size of silver nanoparticle. When pH of the biomass solution was increased from 5 to 9 to 12, this led to a reduction in particle size from 11.9 nm to 11.3 nm and to 8.7 nm respectively.

Using *P.pastoris* biomass as reducing agent, silver nitrate and silver amine were used in separate experiments as sources of silver ions. A comparison was made between these two sources of silver ions under similar reaction conditions, when silver amine was used the bioreduction was much faster and the absorbance peak was higher compared to silver nitrate. When silver amine was source of silver ions, in acidic condition at pH 4 and below there was no bioreduction and increase in pH led to reduction in particle size. When silver nitrate was used as source of silver ions, there was also no bioreduction in acidic conditions at pH 4 and below. Increase in pH led to steady increase in absorbance and a blue shift in the position of absorbance peak from 423 nm at pH 5 to 418 nm at pH 12. Increasing the rotate rate and temperature led to a steady increase in the absorbance and there was no shift in position of the absorbance peak. Increasing biomass concentration led to increase in particle size from 11.98 nm to 12.97 nm. Increasing the ratio of silver ions to biomass led to increase in absorbance and broadening of peak hence suggesting aggregation of nanoparticles in solution.

KEY WORDS: Biosynthesis; Nanoparticles; Silver; *Aleurites moluccana*; *Pichia pastoris*

摘 要

由于纳米银的性质和它的粒径、粒径分布和形貌息息相关，因此如何调控纳米银的粒径和形貌引起了研究者的广泛关注。本研究选取了两种生物质——石栗叶和毕赤酵母菌粉作为还原剂来制备纳米银，研究了反应时间、温度、搅拌速度、生物质/银离子的比值和 pH 值等条件对纳米银的粒径和形貌的影响，并利用紫外-可见分光光度计（UV-Vis）、扫描电子显微镜（SEM）、透射电子显微镜（TEM）和傅立叶变换红外光谱仪（FTIR）等多种分析方法来表征所形成的纳米银。

利用石栗叶作为还原剂，80 ℃时所制备的纳米银的平均粒径大约为 31.1 nm，当温度升高到 100 ℃时，所合成的纳米银的平均粒径减小为 13.2 nm。增大搅拌速度使得纳米银的紫外-可见吸收峰的位置发生了蓝移，峰的强度减弱，当搅拌速度从 10 rpm 增大到 30 nm，所获得的纳米银的平均粒径有轻微减小（26.3 nm→25.5 nm）。而增加石栗叶的浓度则会使得紫外-可见吸收峰的峰强变大，峰的位置发生了蓝移，这说明了随着石栗叶浓度的增加，所制得的纳米银的粒径逐渐减小。此外，我们发现了 pH 值对于纳米银的粒径也有很大的影响，当溶液的 pH 逐渐增大时（5→9→12），所制得的纳米银的粒径逐渐减小（11.9 nm→11.3 nm→8.7 nm）。

利用毕赤酵母菌粉作为还原剂时，本研究选取了硝酸银和银氨来作为银前驱体，比较了二者的差异，研究发现利用银氨作为前躯体时的还原速率要比用硝酸银时快。利用硝酸银作为前驱体时，当 pH≤4 时，溶液中没有纳米银生成；当 pH＞4 时，随着 pH 值的增大，所获得的纳米颗粒的粒径逐渐减小。而利用银氨作为前躯体时，pH≤4 时，溶液中同样没有纳米银生成，随着 pH 值的增大（5→12），纳米银的紫外-可见吸收峰逐渐蓝移（423 nm→418 nm），说明了纳米银的粒径逐渐减小。随着搅拌速度的增大和温度的提高，纳米银的紫外-可见吸收峰的强度逐渐增大，但峰的位置没有发生变化，说明搅拌速度和温度对纳米银的粒径影响不大。银离子浓度一定时，所制得的纳米银的平均粒径随着毕赤酵母菌粉量的增大有轻微的增大（11.98 nm→12.97 nm）。而生物质浓度一定时，增大硝酸银的浓度，所制得的纳米银的紫外-可见吸收峰强度增大，但半峰宽明显变宽，说明

溶液中纳米银发生了团聚。

关键词：生物合成；纳米颗粒；银；石栗；毕赤酵母

LIST OF ABBREVIATIONS

fcc	Face Centered Cubic
fig.	Figure
FTIR	Fourier Transform Infrared
g	Gram
h	Hour
M	Molar
min	Minute
L	Microlitre
mM	Millimolar
nm	Nanometre
rpm	Revolution Per Minute
SEM	Scanning Electron Microscopy
TEM	Transmission Electron Microscopy
UV-Vis	Ultraviolet-Visible
XRD	X-ray Diffraction
a.u	Arbitrary Units
abs	Absorbance
gp	Group

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